conditions. Here, we report a miniature chamber for high-pressure microscopy [1]. The chamber was equipped with a built-in separator, in which water pressure was properly transduced to that of the sample solution. The apparatus developed could apply pressure up to 150 MPa, and enabled us to acquire bright-field and epifluorescence images at various pressures and temperatures. We demonstrated that the application of pressure acted directly and reversibly on the swimming motility of *Escherichia coli cells*. The present technique should be applicable to a wide range of dynamic biological processes that depend on applied pressures [2, 3].

[1] Nishiyama M. and S. Kojima. 2012. Bacterial Motility Measured by a Miniature Chamber for High-Pressure Microscopy. *Int. J. Mol. Sci.***13**: 9225-9239.
[2] Nishiyama M. *et al.* 2013. High Hydrostatic Pressure Induces Counterclockwise to Clockwise Reversals of the *Escherichia coli* Flagellar Motor. *J. Bactetiol.***195**: 1809-1814.
[3] Okuno D. *et al.*, 2013. Single-Molecule Analysis of the Rotation of F_1-ATPase under High Hydrostatic Pressure. *Biophys. J.***105**:1635-1642.

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Bacterial Flagellar Switching: Hidden Markov Steps Revealed

Henry G. Zot[1], Javier E. Hasbun[2], Nguyen Van Minh[3].
[1]Biology, University of West Georgia, Carrollton, GA, USA, [2]Physics, University of West Georgia, Carrollton, GA, USA, [3]Mathematics, Columbus State University, Columbus, GA, USA.

Here we describe a Markov chain that accounts for hidden steps in the switching mechanism of bacterial flagellar rotation. The bacterial flagellar motor apparatus switches between counter clockwise rotation (CCW) and clockwise rotation (CW) by a process that requires interactions between ligand-bound switch subunits of the rotor and motor units of the stator. If the ligand, CheY, binding to switch subunits shifts equilibrium between CCW and CW, i.e. simplest proposed mechanism, the intervals between switches, dwell times, should be a random variable of an exponential distribution. Because dwell times are actually gamma distributed, the switching mechanism has evidence for multiple Poisson steps, which is characteristic of a Markov process. We are proposing that the hidden steps of the switching mechanism are intermediates of the principle thermodynamic states, CCW and CW, generated by rotation. At steady-state, the intermediates factor out of the derived state function, but both the thermodynamic states and intermediates appear as statistical states of a Markov chain. The state function determines the bias and the conditional probabilities for all steps of the Markov chain except when rotation separates a switch subunit, which must have unit probability. To obtain a continuous time random walk, we applied the rotation rate to all conditional and unit probabilities of the discrete Markov chain. Published dwell time distributions were fit best by simulations that depend on bias alone regardless of the number of motor units operating on a rotor. Because the bias in our model depends on the probability that all motor units of a rotor interact with switch subunits that are ligand bound, each motor unit contributes less to the free energy change required for a given bias as motor units increase. Yet the probability of a switch remains constant.

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Mechanical Stress Changes the Movements and Organization of Biofilm-Associated Bacteria

David J. Lemon[1], Xingbo Yang[2], Pragya Srivastava[2], M. Cristina Marchetti[2], Anthony Garza[1].
[1]Department of Biology, Syracuse University, Syracuse, NY, USA, [2]Department of Physics, Syracuse University, Syracuse, NY, USA.

Historically, studies analyzing collective movements, biofilm formation, and the emergence of pathogenicity in bacteria have focused on chemical signals that elicit changes in cell behavior, while responses to physical cues have only recently begun to garner attention. In contrast, the effects of changes in mechanical properties of the environment on eukaryotic cells have been studied extensively. It is known, for instance, that substrate stiffness can guide the migration of epithelial and endothelial cells and has profound effects on cell shape and division. Our research aims to examine how bacteria and bacterial colonies respond to changes in the physical properties of their environment (mechanical stress, stiffness, and surface topography) and to understand the molecular mechanisms behind the response. Previous work showed that the Gram-negative, biofilm-forming bacterium Myxococcus xanthus responds to tension and compression of its substrate's surface by forming elliptical colonies that expand most rapidly perpendicular to the axis of compression. This behavior, dubbed "elasticotaxis", was initially hypothesized to be important for locating food sources. By combining physics-based modelling of the stresses in compressed substrates with experimental data showing the corresponding change in shape of M. xanthus colonies, we have established a linear correlation between the mechanical stress in the substrate and the degree of the elasticotaxis response. To identify the physical changes in the substrate that elicit the elasticotaxis response, we are investigating whether compression of the agar substrate leads to changes in topography, such as wrinkling, or to changes in material properties, such as polymer alignment. Recently, we found that elasticotaxis is not unique to M. xanthus; our preliminary results suggest many bacteria exhibit this behavior. Accordingly, we are examining whether elasticotaxis is common to both gliding bacteria and swarming bacteria and how it may affect biofilm formation.

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Atomic Force Microscope Spectroscopy: Progress toward Antibiotic Resistance and Biofilm Studies

Mehrdad M. Tajkarimi[1], Albert M. Hung[2], Scott H. Harrison[3], Joseph L. Graves[2].
[1]Physical sciecne, Forsyth Tech, Winston-Salem,, NC, USA, [2]Nanoengineering, North Carolina A&T State University, Greensboro, NC, USA, [3]Biological science, North Carolina A&T State University, Greensboro, NC, USA.

The emerging field of live cell nanoscopy and force spectroscopy could revolutionize the way biologists explore the living cell at a molecular resolution. Atomic Force Microscope (AFM) and force spectroscopy analysis have been used to directly measure reversible physiochemical and specific binding interactions between cells. Stickiness is important biofilm formation stage that could also be measured at nN level. A significant source of foodborne illness results from biofilms. These are caused by microorganisms that attach to surfaces and grow as highly organized multicellular communities. This study examines the impact of silver resistance on bacterial adhesion and its viscoelastic formation. We present the first set of data that evaluates the elastic deformation of a bacterial cell surface upon evolution of silver resistance in E. coli MG1655 using AFM compared to controls in 200 generations. The adhesion stickiness and stiffness mean of the treated (evolved Ag resistant) and non-treated samples were tested at nN level. The evolved samples had a significantly ($P<0.05$) higher stickiness ratio and value (from 0.01 ± 0.04 nN to 0.06 ± 0.02 nN) compared to the controls (non-resistant) strains (from0.01 ± 0.02 nN to0.04 ± 0.02 nN). The highest difference of adhesion force happened on Generation 100. According to images of the bacteria in different generations, we can see some major phenotype changes on the appearance at generation 100. The MIC data for the non-evolved strains of E. coli MG 1655 through 200 generations were also significantly lower 38.58 ± 10.09 mg/l compare to evolved strains 272.25 ± 153.94 mg/l ($p<0.01$). The experiment demonstrates important features of phenotype modulation resulting from the evolution of Ag resistance that will be further studied by this group.

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Depletion-Mediated Pattern Formation in a Growing Bacterial Colony

Pushpita Ghosh[1], Jagannath Mondal[2], Eshel Ben-Jacob[1], Herbert Levine[1].
[1]Center for Theoretical Biological Physics, Rice University, Houston, TX, USA, [2]Chemistry, Columbia University, New York, NY, USA.

Secretion of extracellular polymeric substances into growth medium of bacteria is the hallmark of forming biofilm-like structures. The morphological property of such systems might depend upon the physical interactions of cells with extracellular polymeric substances(EPS). We have studied self-organization of nonmotile rod-shaped bacterial cells growing on solid substrate in presence of self-producing EPS, secreted into the growth medium in expanding colony. In our individual-based simulation model of bacterial cells and EPS, all the particles interact mechanically via repulsive forces by pushing each other away as bacterial cells grow and divide consuming diusing nutrient and produce EPS. We show that mechanical interactions control the collective behavior of the system, particularly, we show that the presence of nonadsorbing EPS leads spontaneous aggregation of bacterial cells by depletion attraction and generates phase separated patterns in a nonequilibrium growing colony. This generic mechanism powered by entropic forces could explain one of the possible ways to spontaneous aggregated structure formation and spatial heterogeneity in a biofilm.

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Bacterial Chemotactic Tumble Angles Reduce Backtracking and Maximize Information Gathering

Jan H. Hoh[1,2], William F. Heinz[1].
[1]Physiology, Johns Hopkins School of Medicine, Baltimore, MD, USA, [2]Royal Institute of Technology, Stockholm, Sweden.

Chemotaxing bacteria gather information from the environment and use that to control the balance between runs and tumbles in order achieve a biased motion toward the source of a chemoattractant. We have examined the role of the tumble angle on how effectively gradients are coupled into a bacterium's trajectory. Chemotaxis was simulated using the ZBP program, and the average tumble angle varied from 0 to 180 degrees in the presence and absence of the normal angle variance and/or rotational diffusion. 100,000 step (0.1 μm/step) trajectories from these simulations where analysed using the k-space information